Systematics and biogeography of snakes of the genus *Boa* in the Lesser Antilles

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Abstract

The genus *Boa* is represented in the Lesser Antilles by a range of fossil, recently extinct, and extant forms occupying adjacent island banks in the center of the archipelago. Our multigene molecular phylogeny indicates reciprocal monophyly for these extant forms, and colonization from South America rather than Central America. The timing of the colonization of the Lesser Antilles by this genus indicates a late Miocene or Pliocene event, which is earlier than the Pleistocene event suggested for the *Corallus* treeboas, which are also represented by two extant nominal species. The compact distribution of *Boa* on adjacent island banks suggests a single colonization and radiation, but this cannot be tested due to widespread extinction of boas across the island banks.

Keywords: *Boa*, *Corallus*, multigene phylogeny, Lesser Antilles, biogeography, colonization, radiation.

Introduction

There are six multi-species snake radiations in the Lesser Antilles as well as several genera represented by single species (Thorpe 2022). These have colonized the Lesser Antilles either from the Greater Antilles in the north, e.g., the Lesser Antillean racers (*Alsophis*), or from the mainland, generally South America, in the south e.g., false coral snakes and groundsnakes (*Erythrolamprus*). Some of these have radiated into a sufficient number of species across several island banks to suggest a relatively early colonization, for example the east Caribbean blindsnakes (*Antillotyphlops*, 6 species), *Alsophis* (7 extant species as well as fossil species), and *Erythrolamprus* (four described species). On the other hand, the treeboa *Corallus* has only two nominal species which occupy just the two island banks closest to South America. Moreover, molecular phylogenies show the node of these sister species to be imbedded with the South American species, *C. hortulana*, and not very divergent (Colston *et al.* 2013). This indicates a relatively recent colonization and Colston *et al.* (2013) suggest a Pleistocene over-water colonization.

The situation within the genus *Boa* in the Lesser Antilles is more complicated. In the Lesser Antilles, this genus is composed of older fossil species, more recently extinct species, and extant species. These extant and extinct species occupy six central latitude banks from the Antigua Bank down to the St. Lucia Bank (Fig. 1). While fossil evidence indicates that *Boa blanchardensis* on the Marie-Galante Bank became extinct before human colonization (Bochaton *et al.* 2021), other extinct forms on Antigua (Steadman *et al.* 1984), Basse-Terre (Bochaton *et al.* 2021), La Désirade (Bochaton *et al.* 2021), and Martinique (Dewynter *et al.* 2019; S. Grouard, pers. comm.), and perhaps also Grande-Terre (Bochaton *et al.* 2021), only became extinct recently, i.e. after Amerindian colonization.

Given the overall distribution of the genus *Boa* in South and Central America (Fig. 1), colonization of the

Lesser Antilles from the mainland, rather than the Greater Antilles to the north, is indicated. However, the source, number and timing of colonization events is unclear. There are three species of *Boa* on the mainland, *B. sigma* in western Mexico, *B. imperator* in Central America and Eastern Mexico, and *B. constrictor* in South America. The geographic source of the colonization of the extant Lesser Antillean species, i.e., Central or South America, is yet to be established, as is an estimate of the timing of the event(s). Card *et al.* (2016) investigated the molecular phylogeny of South American *Boa* forms but did not include the Lesser Antillean species in the study.

Although we cannot estimate the molecular phylogenetic relationships of the extinct forms, here we aim to use a multigene approach to assess the relationship between the extant Lesser Antillean species of *B. nebulosa* from Dominica and *B. orophias* from St. Lucia and their relationship with *Boa* species from the mainland (Fig. 1). We also aim to compare the relative timing of the colonization of the Lesser Antilles of the extant *Boa* radiation with that of the other Lesser Antillean boas, i.e. the treeboas of the genus *Corallus*.

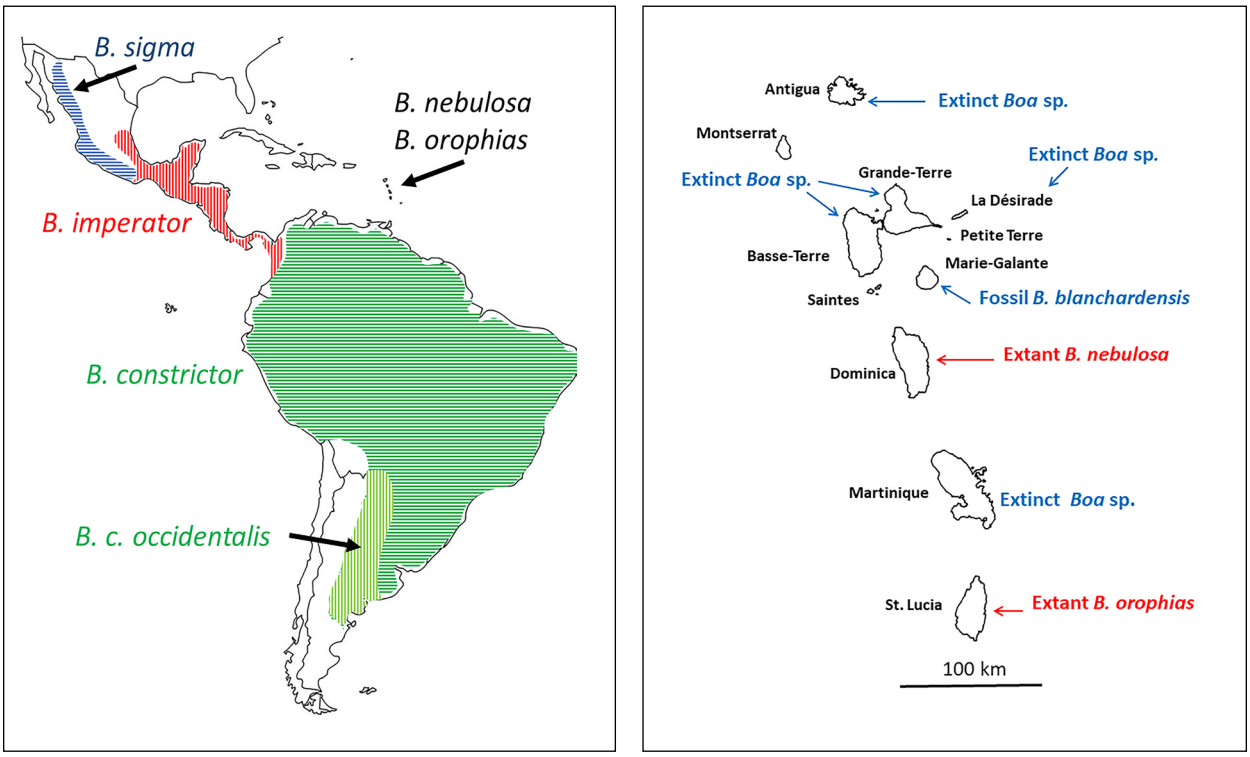

Figure 1. Distribution of the genus *Boa*. **(A)** (Left) Approximate distribution of extant *Boa* species. **(B)** (Right) Distribution of extinct (blue) and extant (red) *Boa* species in the more central Lesser Antilles.

Methods

DNA extraction and PCR amplification. DNA extraction from a single Dominican *Boa* sample (taken from a road-kill) was performed using the Qiagen DNeasy® Blood and Tissue Kit following the manufacturer's protocol (Qiagen 2020). Gel electrophoresis and a Nanodrop Spectrophotometer ND1000 were used for checking the concentration and quality of extracts. Four mitochondrial genes, cytochrome b (Cytb), NADH dehydrogenase subunit four (ND4), 12S small subunit ribosomal RNA (12S), and 16S large subunit ribosomal RNA (16S) and one nuclear gene, neurotrophin 3 (NT3) were PCR-amplified using Thermo Fisher Scientific DreamTaq Mastermix. The primers and their annealing temperatures used for each gene are listed in Table 1. The PCR cycling conditions were an initial denaturation at 94 °C, followed by 35 cycles of 94 °C for 30 s, an annealing phase at the appropriate temperature for each set of primers for 30s, and an extension at 72 °C (of 1 min for the longer products, i.e., Cytb and ND4, and 45

s for the shorter products i.e., 12S, 16S, and NT3), and a final extension at 72 °C for 5 minutes. Successful amplification was confirmed using gel electrophoresis and PCR products were cleaned for sequencing using Thermofisher ExoSAP-IT and Sanger-sequenced at Macrogen Europe (Amsterdam, Netherlands).

Table 1. PCR primers used in this study along with their sequences. F and R indicate the forward and reverse primers respectively, and the annealing temperature is given for each pair.

Gene	Primer name (F/R)	°C	Sequence (5′ to 3′)	Reference
Cyt b	Gludgmod (F)	55	CTT GAA AAA CCA CCG TTG T	Dawson *et al.* 2008
Cyt b	H16064mod (R)	55	GGT TTA CAA GAA CAA YGC T	Dawson *et al.* 2008
ND4	ND4 (F)	57	CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC	Arevalo *et al.* 1994
ND4	LEU (R)	57	CAT TAC TTT TAC TTG GAT TTG CAC CA	Arevalo *et al.* 1994
12S	L1091 (F)	45	AAA CTG GGA TTA GAT ACC CCA CTA T	Knight & Mindell 1993
12S	H1557 (R)	45	GTA CAC TTA CCT TGT TAC GAC TT	Knight & Mindell 1993
16S	L2510 (F)	50	CGC CTG TTT ATC AAA AAC AT	Palumbi 1996
16S	H3059 (R)	50	CCG GTC TGA ACT CAG AT	Palumbi 1996
NT3	NTF_SC_F	58.5	GCA TTT CTG TGT GGC ATC CA	unpublished
NT3	NTF_SC_R	58.5	CGA GGT TTT GCA CTG GGA AT	unpublished

Alignment and phasing. Resulting chromatograms were edited in MEGA7 (Kumar *et al.* 2016) to correct any errors, trim the ends, and detect heterozygous positions present in the nuclear gene sequence. Heterozygous base pairs were assigned the appropriate IUPAC ambiguity code. Additional sequences of *Boa* and other representative Neotropical boine genera were downloaded from GenBank, selecting those which had multiple genes represented, and those for which locality information was available. Most specimens only had Cytb available, so multiple gene alignments were made: A. Cytb only, containing a larger number of specimens, and B. Four mitochondrial genes concatenated, with fewer specimens (in some cases, gene sequences from samples from similar locations were combined), C. NT3 sequences (very few specimens). In all cases, *Eryx tartaricus* (Boidae: Erycinae) was selected as the outgroup. NT3 haplotypes were reconstructed using PHASE in DnaSP v6 (Rozas *et al.* 2017) using default settings (except no recombination was assumed since only a short section of the gene was used).

Phylogenetic analysis of mitochondrial genes. *Maximum likelihood*: We inferred a maximum-likelihood (ML) tree using the edge-linked partition model in IQ-TREE (Chernomor *et al.* 2016; Nguyen *et al.* 2015) on the IQ-Tree web-server (Trifinopoulos *et al*. 2016) for both the Cytb and concatenated mitochondrial datasets. Data were fully partitioned, resulting in 3 partitions for the Cytb dataset and 8 partitions (12S, 16S, and the three codon positions separately for the two coding genes Cytb and ND4) for the concatenated mitochondrial dataset. Models selected under the Bayesian Information Criterion (BIC) by ModelFinder (Kalyaanamoorthy *et al.* 2017) which is implemented in IQ-Tree, with 10,000 UltraFast Bootstraps (UFB) (Minh *et al.* 2013) and approximate Shimodaira-Hasegawa Likelihood Ratio branch tests (SH-aLRT) (Guindon *et al.* 2010). Trees were visualised using Figtree v1.4.4 and edited for clarity in Dendroscope v3.5.10 (Huson & Scornovacca 2012).

Calibrated Bayesian timetree: The freeware phylogenetic analysis package BEAST2 (Heled & Drummond 2010), was used to co-estimate a gene phylogeny and associated divergence times with calibration times from fossil evidence. For this, we used the concatenated mitochondrial dataset and a fully partitioned scheme as above, using the most similar model available to those selected by IQ-tree. Some individuals were concatenated to maximise representation in this analysis, details can be found in Table 2. The genes trees and clocks were linked, but evolutionary models were left unlinked. The clock was set to Relaxed Clock Log Normal and the Calibrated Yule Model prior was selected. Two nodes were calibrated with dates derived from fossil data (Head 2015): (1) Boinae, modelled as a log-normal distribution with an offset in real time of 58.0 my, with a mean of 2.0 and S of 0.7 (selected to give a 97.5% quantile estimate of c. 64 my for the soft maximum bound) and (2) The divergence between

Table 2. The specimens used in this study and their GenBank accession numbers. Some specimens were combined for the concatenated analysis. Locality codes are standard 2-letter ISO codes for country and main division (state/province/district/parish). Species names are as given in GenBank. NA = not applicable.

Voucher	Taxon	Locality code	CYTB	ND4	12S	16S	NTF3	Additional locality details	Comments
Specimens used in concatenated analysis									
B351	*Boa nebulosa*	DM_PL	OR352218	OR352219	OR351921	OR351929	OR352220	Corona Estate, near Pond Casse	-
NE1.7	*Boa nebulosa*	DM	KF576731	KF576696	KF576860	KF576948	-	-	-
NE6.7	*Boa nebulosa*	DM	KF576733	KF576699	KF576863	KF576951	-	-	-
NE1.12	*Boa orophias*	LC	KF576734	KF576700	KF576864	KF576952	-	-	-
NE5.13	*Boa constrictor*	-	KF576737	KF576704	KF576868	KF576956	-	*occidentalis* (Argentine boa)	-
NE6.11	*Boa constrictor*	-	KF576738	KF576705	KF576869	KF576957	-	*occidentalis* (Argentine boa)	-
RGR BOCO1	*Boa constrictor*	PR	JX026897	KC329954	-	-	KC330109	introduced	cytb identical to NV26, combined into South America
NV26	*Boa constrictor*	GF_CY	AF471036	-	-	-	-	Petit Saut	-
AEH040	*Boa constrictor*	-	-	-	-	-	AY988047	-	Allan E. Hebert field series
NE1.18	*Boa imperator*	-	KF576739	KF576706	KF576870	KF576958	-	*ortonii* (coastal Peru)	-
NE6.10	*Boa imperator*	-	-	KF576703	KF576867	KF576955	-	*longicauda*, variety of *ortonii* (coastal Peru)	groups with *imperator*
NE1.16	*Boa imperator*	PA_PM	KF576746	KF576714	KF576878	KF576966	-	Pearl islands	-
NE6.9	*Boa imperator*	PA_PM	KF576747	KF576715	KF576879	KF576967	-	Pearl islands	-
MVZ162363	*Boa imperator*	PA_PM	AB177354	AB177354	AB177354	AB177354	-	Barro Colorado Island	-
USNMFS 254018	*Boa imperator*	PA_CC	-	-	-	MH140639	-	Penonome, ca. 7 km E of, on Inter-American Highway	-
UTA-R24752	*Boa imperator*	-	-	-	AF512744	AF512744	-	Central America	-
US-	*Boa imperator*	PA_BC	KJ621526	-	-	MH140638	-	Isla Escudo de Veraguas, West Point	-
IQU1	*Boa constrictor*	PE_LO	EU273623	-	-	-	-	Iquitos	-
PERW	*Boa constrictor*	PE_SM	EU273635	-	-	-	-	Tarapoto	-
Bco1	*Boa constrictor*	-	HQ399514	-	-	-	HQ399534	*occidentalis* (Argentine boa)	-

Cann45/ CAF5	*Corallus annulatus*	CR	HM348832	-	JX244285	-	JX244325	-	combined with Cann1 for mtDNA
Cann1	*Corallus annulatus*	-	KC750011	KC750017	-	-	KC750023	-	combined with Cann45 for mtDNA
CH5152	*Corallus annulatus*	PA_CC	-	-	-	MH140664	-	La Pintada, El Harino	-
Cbat1/ RGRCorBat1	*Corallus batesii*	BR_AM	KC750013	-	-	-	KF811133	-	both specimen numbers used by Reynolds.
Ccann1	*Corallus batesii*	BR_AM	-	KC750019	-	-	-	-	-
54/ MPM23596	*Corallus cooki*	VC	HM348836	-	JX244287	-	JX244326	-	-
CTMZ00327	*Corallus cropanii*	BR_AM	JX576180	-	JX576163	-	JX576183	Amazon Basin	-
57/ MPM25665	*Corallus grenadensis*	GD_AN	HM348837	-	JX244288	-	NA	-	-
24/LSUMZ	*Corallus hortulana*	EC_SU	HM348857	-	JX244289	-	NA	-	GB record says Rondonia but table in paper says Ecuador
55/IB55042	*Corallus hortulana*	BR_SP	HM348879	-	JX244295	-	JX244329	Iguape	-
31/KUWED 57796	*Corallus hortulana*	PE_CS	HM348864	-	JX244292	-	JX244327	-	GB record says Amazonas but table in paper says Cusco
52/CRF1	*Corallus ruschenbergerii*	TT	HM348842	-	JX244298	-	JX244331	-	-
RGRmur1	*Eunectes murinus*	-	KC329952	KC329977	JX576166	-	KC330140	-	12S from different specimen (MZUSP13966)
Arg3	*Chilabothrus argentum*	-	NC_063114	NC_063114	NC_063114	NC_063114	NA	-	-
RGR MDP1	*Chilabothrus inornatus*	PR_AC	KC329929	KC329962	-	-	KC330117	-	-
RGR GRT1	*Chilabothrus monensis*	VI_ST	KC329931	KC329964	-	-	KC330119	-	-
CAS 231783	*Epicrates maurus*	TT_SL	KC329951	KC329976	-	-	KC330139	Blanchisseuse	-
CH265/ NE6.21/ UMFS11688	*Eryx tataricus*	CN_XG	MN646174	MN646174	MN646174	MN646174	-	-	combination of several specimens
Specimens used only in cytb analysis									
NE1.8	*Boa nebulosa*	DM	KF576732	-	-	-	-	-	-
NE1.15	*Boa orophias*	LC	KF576735	-	-	-	-	-	-
NE6.6	*Boa orophias*	LC	KF576736	-	-	-	-	-	-
BRA2	*Boa constrictor*	BR_PA	EU273653	-	-	-	-	Marajó	-

LONG3	*Boa constrictor*	PE	EU273621	-	-	-	-	-	listed as *B. c. longicauda* but groups with *B. imperator*
CC1	*Boa imperator*	BZ_BZ	EU273606	-	-	-	-	Crawl Cay	-
BCCA	*Boa imperator*	BZ_BZ	EU273608	-	-	-	-	Crawl Cay	-
BCK1	*Boa imperator*	CO_CH	EU273609	-	-	-	-	BieChoco	-
BCK0	*Boa imperator*	CO_CH	EU273611	-	-	-	-	BieChoco	-
hoog	*Boa imperator*	HN_IB	EU273613	-	-	-	-	Hog island	-
NIC1	*Boa imperator*	NI	EU273615	-	-	-	-	-	-
YUC1	*Boa imperator*	MX_QR	EU273616	-	-	-	-	Cancún	-
SAL01	*Boa imperator*	ES_AH	KJ621532	-	-	-	-	San Francisco Menéndez	-
CHIA1	*Boa imperator*	MX_CP	EU273619	-	-	-	-	Tuxtla Gutiérrez	-
TAM05	*Boa imperator*	MX_TM	KJ621474	-	-	-	-	Barra del Tordo	-
VER02	*Boa imperator*	MX_VE	KJ621477	-	-	-	-	Los Chimalapas	-
PAN05	*Boa imperator*	PA_PM	KJ621529	-	-	-	-	Paraíso	-
PAN01	*Boa imperator*	PA_BC	KJ621526	-	-	-	-	-	-
GUA1	*Boa imperator*	GT_ES	EU273620	-	-	-	-	-	-
SAB2	*Boa imperator*	PA_PM	EU273665	-	-	-	-	Saboga island	-
COS4	*Boa imperator*	CR_LI	GQ300924	-	-	-	-	Cahuita	-
no voucher	*Boa sigma*	MX_MC	AY575035	-	-	-	-	-	-
SON01	*Boa sigma*	MX_SO	KJ621416	-	-	-	-	Álamos	-
SIN03	*Boa sigma*	MX_SI	KJ621419	-	-	-	-	Acaponeta	-
COL01	*Boa sigma*	MX_CL	KJ621420	-	-	-	-	El Naranjo	-
JAL01	*Boa sigma*	MX_JA	KJ621423	-	-	-	-	Puerto Vallarta	-
JAL09	*Boa sigma*	MX_JA	KJ621431	-	-	-	-	Mascota	-
MICH03	*Boa sigma*	MX_MC	KJ621437	-	-	-	-	Solera de Agua	-
GRO01	*Boa sigma*	MX_GR	KJ621442	-	-	-	-	Ayutla	-
OAX01	*Boa sigma*	MX_OA	KJ621445	-	-	-	-	Puerto Escondido	-
Specimens used only in the NT3 analysis									
Bimp1/ RGR BOIM1	*Boa imperator*	-		-	-	-	KF811128	-	-

Corallus and *Eunectes*+*Chilobothrus*+*Epicrates* was modelled as a log-normal distribution with an offset in real time of 50.2 my, with a mean of 2.0 and S of 2.0 (selected to give a 97.5% quantile estimate of c. 64 my for the soft maximum bound).

The MCMC chain length was set to 100,000,000 with a sampling frequency of 5,000. The resulting xml file was run twice with different random seeds. To check that the calibrations on the nodes were not overly influencing the topology, the analysis was also run "on empty" by sampling from the priors. Trace log files were analysed using Tracer v1.7. Tree files were combined with LogCombiner using an appropriate, burn-in cut-off (determined by the log likelihood trace reaching stationarity), and the resulting species tree file was summarised using TreeAnnotator using default values (Barido-Sottani *et al.* 2018). The resulting tree was visualised using Figtree v1.4.4.

Nuclear haplotype networks. Haplotype networks were created for NT3, as the low level of variation allows mutational steps between haplotypes to be reconstructed. A Median-joining network (Bandelt *et al.* 1999) was created and visualised using PopART (Leigh & Bryant 2015).

Results

Final aligned datasets consisted of 1062 bp of Cytb, 708 bp of ND4, 530 bp of 16S, 424 bp of 12S, and 467 bp of NT3. The cytb dataset consisted of 63 specimens while the concatenated mtDNA dataset contained 27 specimens (Table 2). No heterozygous base pairs were found in the NT3 dataset, and 16 specimens were included in the analysis.

Maximum likelihood. The cytochrome b ML phylogeny corresponded with expectations, showing well-supported clades corresponding to the relatively newly defined species *Boa sigma* and *Boa imperator* (Fig. 2). However, two specimens from South America (according to the assigned names), which according to Card *et al.* (2016) are part of the *B. constrictor* clade instead appear within the *B. imperator* clade. According to the subspecies designation, these are from coastal Peru (*B. c. ortonii*, *B. c. longicauda*): hence, these have either been misidentified, cross-bred, or the distribution of *B. imperator* extends further south along the west coast of South America than recognised by Card *et al.* (2016), but which has been previously highlighted by Hynková *et al.* (2009). However, other samples from Peru fall within the expected cluster of *Boa constrictor* samples. Although all specimens of *B. constrictor* did not form a monophyletic clade (in particular, a group of samples of the Argentinian boa *B. c. occidentalis* were quite distinct), it is important to recognise that this phylogeny is based on a single gene only, and that many of the deeper nodes are not well supported. The position of *B. orophias* and *B. nebulosa*, the Lesser Antillean populations, is however clear: they are reciprocally monophyletic sister taxa, with their affinities quite clearly with Southern American populations (note that the sample from Puerto Rico is from an introduced population, which is identical to a specimen from French Guyana).

There were no *B. sigma* samples included in the concatenated mitochondrial ML phylogeny, as Cytb is the only gene represented on GenBank for this species. There is better definition of the two major clades (*B. imperator* and *B. constrictor*) and the Lesser Antillean populations are clearly embedded within the *B. constrictor* clade (Fig. 3). With regard to the other boines, it is notable that in both of the above phylogenies, there is very little differentiation among the *Corallus hortulana* specimens from across their range, including the two nominal species from the Lesser Antilles.

Calibrated phylogeny. The two independent runs converged on identical likelihoods and topologies, and the run sampling from priors only showed no shift from prior distributions, indicating that the calibration was not affecting the posterior distributions. All ESS values were well above the minimum of 200, and a cut-off of 10% was adequate to exclude the burn-in iterations. Nodes were all well supported (PP>95%) except for some internal nodes within *Boa nebulosa* and within the *Coraluus hortulana* complex (Fig. 4). Unexpectedly, *Corallus batesii* was strongly supported as sister to *Epicrates*, which is consistent with its low support as part of the *Corallus* clade in the mtDNA tree. However, the key clades (*Boa* species and the *Corallus hortulana* complex) were all highly supported

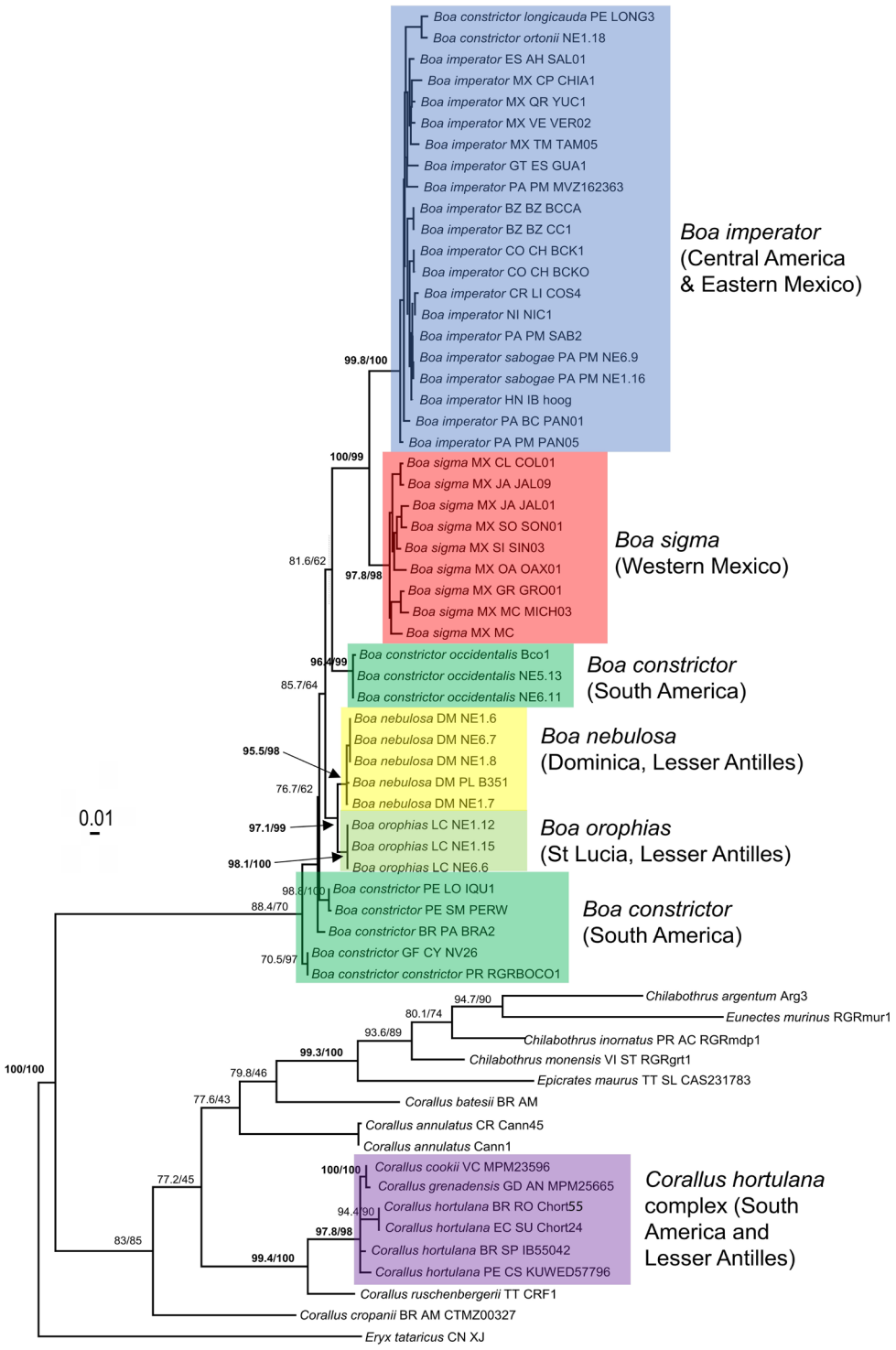

Figure 2. Maximum-likelihood tree based on cytochrome b sequences. Location codes follow the ISO system (country and major division) and are followed by specimen voucher codes. Support values are approximate Shimodaira-Hasegawa Likelihood Ratio (SH-aLRT) tests, with Ultra-fast bootstraps (UFB) after the slash (some intra-clade support values have been removed for clarity). Some support values within major clades have been removed for clarity. A highly supported clade is indicated by SH-aLRT >= 80% and UFB >= 95%. All clades indicated by colored boxes are supported at this level. The scale bar represents the number of nucleotide changes per site.

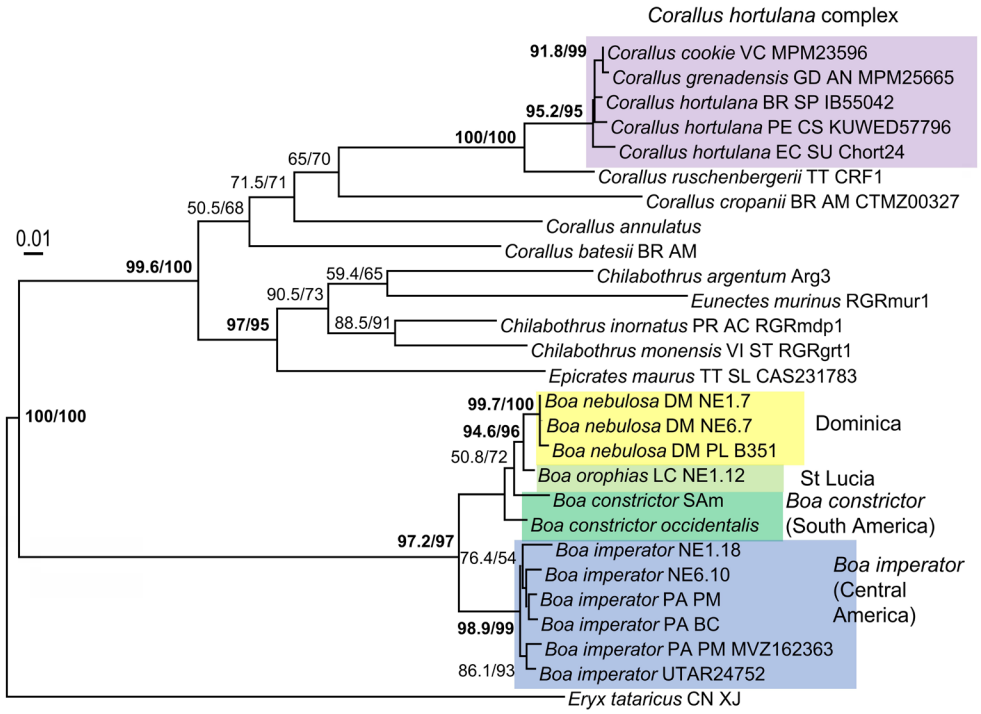

Figure 3. Maximum-likelihood tree based on concatenated sequence of four mitochondrial genes. Values at highly supported nodes are given in bold and support values at some unsupported nodes have been deleted for clarity. Other formatting as in Fig. 2.

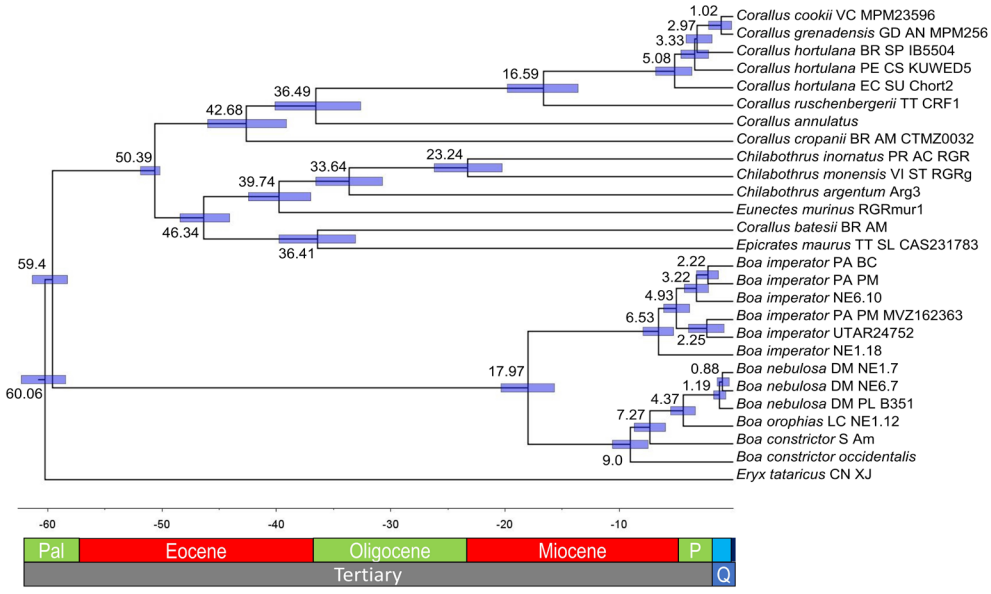

Figure 4. Calibrated Bayesian tree based on concatenated sequence of four mitochondrial genes. All nodes are supported by posterior probabilities >= 95%, except for some within-species nodes. Values at the nodes represent the mean estimate of divergence time at that node (in millions of years). Bars at nodes represent 95% HPD (*Boa nebulosa* + *B. orophias*: 3.35–5.49 my; *B. constrictor*: 5.95–8.7 my; *B. imperator*: 5.24–7.9 my; *B. constrictor* + *B. imperator*: 15.67–20.33 my; *Corallus hortulana* complex: 3.65–6.81 my; *Corallus* (excluding *C. batesii*): 39.14–46.01 my; *Chilabothrus*: 30.73–36.56 my). The scale indicates time from the present (in millions of years). In the geological timescale, Q = Quaternary (with light blue representing the Pleistocene and dark blue the Holocene), P= Pliocene, Pal= Paleocene. Other formatting as in Fig. 2.

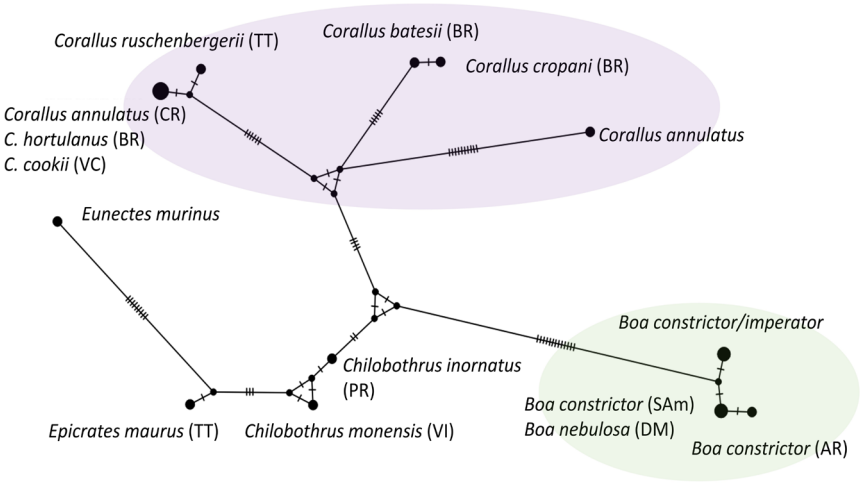

Figure 5. NT3 median-joining network for Neotropical boines. Vertical hatches represent number of mutations separating haplotypes. The size of the circle represents the number of identical haplotypes sampled while the smallest dots represent inferred haplotypes. Two-letter codes are ISO country codes (Except SAm = South America).

monophyletic clades (Fig. 4). The radiation of *Boa* occurs considerably later than *Corallus* as a whole (excluding *C. batesii*), at 18.0 compared to 42.7 mya. However, the split between the Lesser Antillean members of both clades is very recent, with Lesser Antillean *Boa* diverging from the mainland specimens 7.27 mya and the split between *B. nebulosa* and *B. orophias* occurring only 4.37 mya. In comparison, the divergence between the Lesser Antillean *Corallus* and the South American populations occurred 2.97 mya, with the split between *C. cookii* and *C. grenadensis* dated at only 1.02 mya.

NT3 network. The paucity of available NT3 sequences limits the interpretation of the nuclear network, but it also reflects the much larger differences within *Corallus* compared to *Boa* (Fig. 5). In both *Corallus* and *Boa*, the Lesser Antillean populations share haplotypes with South American representatives.

Discussion

While the focus of this paper is the genus *Boa* in the Lesser Antilles, the molecular phylogeny of the outgroup includes other taxa of interest. The divergence of the Lesser Antillean *Corallus* populations is compatible with colonization of the Lesser Antilles in the Pleistocene estimated in Colston *et al.* (2013). However, Reynolds *et al.* (2013) estimated a much younger divergence time for the origin for *Chilabothrus*, based on 10 genes (including 7 nuclear genes), at 21.7 mya (95% HPD 16.9–26.0 mya) compared to our estimate of 42.68 mya (95% HPD 30.73–36.56 mya). Nevertheless, in the same analysis the divergence between *Boa* and the combined *Corallus*, *Eunectes*, *Epicrates* and *Chilabothrus* clade was estimated at 60.5mya (95% HPD 58.1– 65.4 mya), which is consistent with our estimate of 59.4 (95% HPD 58.26–61.36 mya). The difference may be a consequence of the different number and species of *Chilabothrus* included, as the emphasis of the two phylogenies is different. Alternatively, it may be from different calibrations used. In particular, Reynold's *et al.* (2013) found that including the calibration for the split between *Corallus* and other neotropical boids of 58–61 mya yielded older ages across the boid tree than if it was not included. They concluded that "further study is warranted to determine the accuracy of this fossil calibration and the influence of additional calibrations on age estimates…..in the boid tree."

The Marie Galante boa, *B. blanchardensis*, appears to have become extinct in the late Pleistocene (Bochaton *et. al*. 2021), while the Antigua, Guadeloupe Bank, and Martinique boas appear to have existed at least until

Amerindian times (Steadman *et al.* 1984, Bochaton *et al.* 2021, Grouard *personal communication* in Thorpe 2022). However, while the fossil and subfossil record of *Boa* species in the Lesser Antilles allows an estimate of the timing of their extinction, it does not allow an estimate of the time or sequence of their original colonization.

The reciprocal monophyly of the Dominica and St. Lucia boas indicates a single colonization and subsequent divergence for at least these two species, with the candidate source being *B. constrictor* from South America, rather than the Central American species. This is rather similar to the Fer de Lance, *Bothrops*, in the Lesser Antilles, which has two extant, reciprocally monophyletic species (St. Lucia and Martinique) with their closest relatives in northern South America (Wüster *et al.* 2002). Martinique is positioned between Dominica (*B. nebulosa*) and St. Lucia (*B. orophias*) and its recently extinct *Boa* is therefore most likely part of the same radiation. Given that the other extinct and fossil species of *Boa* in the Lesser Antilles are all in the adjacent central islands from Antigua down to St. Lucia (excluding Montserrat and Les Saintes) one may also speculate that these are part of the same initial colonization and radiation. With an estimated length of ~1.4 m, *B. blanchardensis* from Marie Galante may be smaller than the females of the extant Lesser Antillean boas, but Card *et al.* (2016) has shown that size is evolutionarily labile, so that does not exclude it from being part of the same radiation.

Other squamate groups, such as groundsnakes (*Eryrthrolamprus*) and Antillean two-lined skinks (*Mabuya*), have a similar distribution pattern in the Lesser Antilles suggesting a colonization from South America directly to the central islands rather than a south-to-north stepping-stone route through the archipelago. Indeed, a similar route is now thought to be the case with earliest human colonization (Fitzpatrick, 2015).

While it is thought that treeboas (*Corallus*) colonized the Lesser Antilles from South America very recently, i.e., 0.3–1.2 mya (Colston *et. al*. 2013), it is clear that the *Boa* colonization was earlier. i.e., from the late Miocene to the Pliocene. The mean date of divergence between the two extant Lesser Antillean species (*B . nebulosa* and *B. orophias*) is 4.37 mya (Fig. 4). Notwithstanding that lineage coalescence times will predate physical isolation this is most likely a considerable underestimate of the time of colonization of the Lesser Antilles by the genus. This is because most *Boa* taxa in the Lesser Antilles are extinct and not represented in this molecular phylogeny, so their

Figure 6. (Left) *Boa nebulosa* (head inset) from Dominica.
Figure 7. (Above) A breeding ball of *Boa orophias* (head of female inset) from St. Lucia (Thorpe 2022).

divergence is not assessed. On the other hand, the divergence between *B. constrictor* and the Lesser Antillean clade of 7.27 mya may be an over-estimate as unsampled haplotypes in either clade may exaggerate their divergence.

The ability to colonize across the Lesser Antillean archipelago differs among squamate taxa (Thorpe 2022), but if this timing is reliable and there was a single colonization and radiation, then *Boa* was effective in spreading between seven island banks from Antigua to St. Lucia in a relatively short time. Other studied radiations e.g. *Dactyloa* and *Ctenonotus* (excluding the *wattsi* group) are much older; for example, the former has a 32–44 mya, and the latter a 22–44 mya, within-clade divergence within the Lesser Antilles (Thorpe *et al.* 2018).

Bonny (2007) first recognised both extant Lesser Antillean *Boa* as distinct species, *B. nebulosa* and *B. orophias*. More recent publications e.g., Hedges et. al. (2019) and Thorpe (2022) continue to recognise the species status of both forms and this study does not contradict this (Fig. 6-7). In contrast, the two nominal treeboa species of *Corallus cookii* (St. Vincent) and *C. grenadensis* (Grenada Bank) are not as highly divergent from the mainland *C. hortulana* (Colston *et al.* 2013) prompting Colston *et. al.* (2013) to remark "mitochondrial DNA sequence divergence among these three taxa is minimal (<2% uncorrected sequence divergence), questioning the validity of the taxonomic status of *C. grenadensis* and *C. cookii*", and that the "taxonomic validity of *C. grenadensis* and *C. cookii* are questionable". Even so, these Lesser Antillean treeboas are widely recognised as distinct species including, most recently, by Thorpe (2022) and Reynolds *et al.* (2023).

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